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Expression and Clinical Significance of Various Cytokines in Otitis Media

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Abstract

The expression and clinical significance of relevant cytokines in otitis media (OM) are discussed, and the alterations to the pathological state of the otitis media mucosa are further understood through the study of cytokine transduction pathways. More and more studies have shown that relevant cell proliferation and inflammation progression pathways play a role in the development of otitis media, such as the Jun amino-terminal protein kinase (JNK) mitogen-activated protein kinase (MAPK) signaling pathway, the NF-κB signaling pathway, and the PI3K/AKT/PTEN pathway, which are involved in the proliferation of the middle ear mucosa during otitis media, which affects the mucosal cilia, motor function, Eustachian tube function, and the mucosal ciliary function. These studies provide new ideas for the treatment of otitis media and further explore the feasibility of immunotherapy in the future treatment of otitis media. In this paper, we present a review of the latest research progress on the expression of various cytokines in otitis media.

Keywords

Otitis Media, Middle Ear Mucosa, Cytokines, Eustachian Tube Function, Ciliary Transport Function

1. Introduction

Otitis media is one of the most common otological diseases that jeopardize human hearing, and despite the better prognosis of otitis media through many surgical and pharmacological treatments both at home and abroad, it is still a common cause of hearing loss and reduces the quality of human life. The World Health Organization (WHO, 2004) estimates that untreated OM causes about

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30,000 deaths per year and is responsible for 50% of severe hearing loss worldwide [1]. Liu Zhaoding *et al.* [2] through epidemiological analysis, the incidence of otitis media in China decreased from 1990 to 2019. 39%, and male incidence was higher than female, but the standard incidence was lower than female; the <5 years of age group accounted for the highest proportion of cases. However, the disease burden remains at a high level compared to the rest of the world, and necessary interventions are still needed to reduce it. Otitis media is multifactorial, and it is currently believed that the most important factors are colonization of the middle ear by pathogenic microorganisms and immune dysfunction, as well as environmental and genetic factors. Studies have shown [3] that the smoking environment can either directly affect the mucus quality and volume by disrupting the change of mucus, interrupting ciliary waving, or destroying ciliary cells in the middle ear and eustatic tube, or indirectly lead to eustatic tube obstruction through chemical stimulation and suppression of immune function, making the body more susceptible to bacterial or viral infections. It is known that formaldehyde, acrolein, ammonia, phenol, and cyanic acid contained in cigarette smoke can inhibit ciliary movement, cause ciliary swelling, cause loss of ciliary epithelium, and increase mucus production, thus changing the quality and quantity of mucous membrane in the middle ear mucosa and interfering with the promotion of mucous blanket. Particulate matter (PM) is an important atmospheric pollutant, and PM exposure triggers an airway inflammatory response involving multiple pro-inflammatory cytokines, including interleukin (IL-1), IL-6, and tumor necrosis factor (TNF-α), leading to oxidative stress in the respiratory and circulatory systems. As well as DNA or cell damage, the potential mechanisms of PM exposure and OM may involve the following aspects: 1) The pollutants directly affect the mucosa of the eustaping tube, leading to edema and excessive secretion of mucus, thus obstructing the eustaping tube; 2) PM disrupts mucociliary clearance and weakens the upper respiratory tract's defense against viral infection, thereby increasing the possibility of disease [4]. Machogu and Gaston [5] found that primary ciliary dyskinesia (PCD) is mainly inherited in an autosomal recessive way. So far, more than 45 pathogenic genes have been identified. It is a clinically heterogeneous disease that can lead to chronic otitis media with hearing impairment, and PCD is characterized by impaired motor cilia function, leading to chronic inflammatory manifestations of the middle ear. Mucopolysaccharidosis (MPS) is a hereditary lysosomal storage disease with multi-system and highly variable clinical manifestations, resulting in inadequate degradation and accumulation of glycosaminoglycans (GAG) in various tissues. The increased risk of otitis media in MPS patients is most likely due to GAG deposition in the posterior nasal cavity and middle ear caused by elevated GAG concentration in the middle ear fluid and frequent adenoid hypertrophy leading to tubal dysfunction [6]. The function of the middle ear is to conduct hearing and maintain stable air pressure in the ear. Chronic otitis media develops when the important functions of the middle ear are disrupted, resulting in hearing loss, ear discharge, and tinnitus, which are closely related to the function of the

Eustachian tube, the function of mucosal cilia transport, and changes in the structure of the associated proteins [7] [8]. This is closely related to alterations in eustachian tube function, mucosal ciliary transport, and protein structure. In this article, we describe the major advances made recently in understanding the contribution of cytokine expression to the development of otitis media and the insights these studies provide into the pathogenesis of otitis media.

2. Overview of Otitis Media

2.1. Middle Ear Physiology

(Padurariu *et al.*, 2019) [9] has been found that the physiological function of the middle ear (ME) is the transmission of sound and the stable maintenance of intra-auricular pressure. Pressure gradients are constantly generated by gas exchange across the mucosa of the middle ear as well as changes in ambient pressure. In the normal middle ear (ME), optimal sound transmission and hearing are ensured by maintaining a balance between pressure and environmental pressure. This equilibrium is influenced by a number of factors, one of which is the continuous bi-directional diffusion of gases between the ME cavity and the mucosal vasculature, and the other is the movement of the tympanic membrane, which counteracts pressure changes from the outside. The mucus cilia system of the Eustachian tube and the innate immune molecules secreted by its epithelial cells create a favorable barrier against invading pathogenic microorganisms and help maintain the sterility of the Eustachian tube and the tympanic chamber. Homeostasis of the Eustachian tube and middle ear is maintained in part by mucins, water channel proteins, surfactants, and antimicrobial molecules, and the production of mucins and the balance of the periciliary fluid are essential for normal mucociliary function, and dysfunction of this system is an important risk factor for otitis media [10].

2.2. Middle Ear Mucosa

Tympanic chamber (TC) mucosal epithelial types are more varied, ranging from complex columnar cilia to a single layer of cuboidal flattened epithelium, but with taller anterior lower cells and shorter posterior upper cells. In addition to cilia, the tympanic mucosa may contain functions related to immune defense and exudate removal (including secretory cells to remove cellular debris). Thus, the middle ear can be divided into the posterior superior chamber (consisting of the superior tympanic chamber, the tympanic sinus, and the mastoid airspace system) and the anterior inferior chamber (consisting of the tympanic cavity), with the posterior superior tympanic chamber primarily performing gas exchange and the anterior inferior chamber primarily involved in scavenging functions and immune defense [9]. Since transmucosal gas exchange is caused by a localized pressure gradient between the middle ear cavity and the mucosal capillaries, this mucosal exchange can be impaired by inflammatory changes in the middle ear mucosa and mastoid process, resulting in hearing loss. The condition

of the middle ear mucosa around the opening of the Eustachian tube is critical for maintaining gas exchange in the middle ear cavity, and edema and inflammatory granulation tissue changes in the middle ear mucosa may obstruct the opening of the Eustachian tube, which is detrimental to the maintenance of the pressure gradient in the middle ear [11]. During otitis media, the middle ear mucosa has the unique ability to grow and proliferate to several times its original thickness, forming highly structured, pseudocomplex, columnar epithelial complexes. Mucosal hyperplasia can also lead to deleterious sequelae of OM, including secretion of mucus into the middle ear and formation of effusion leading to secretory otitis media, glue ear, and vasodilatation of mucosal vessels, which also increases the chances of leukocyte infiltration and tissue edema, and increased stromal cells, which can lead to stromal fibrosis, conjunctival fibrosis, and occlusion of the middle ear. can lead to stromal fibrosis, connective tissue, and neovascularization, which may permanently damage the middle ear's conduction system and lead to persistent hearing loss [12] [13]. The diseased mucosa can act as a barrier to normal gas exchange or directly block the opening of the eustachian tube. Changes in the morphology of the middle ear mucosa indicate that the disease is in an active state. Therefore, if there is swelling or hyperplasia of the middle ear mucosa, it may mean poor ventilation in the middle ear, indicating that the disease is in an active stage [11].

3. Promotes Mucosal Proliferation and Facilitates Inflammatory Signaling Pathways

3.1. JNK Induced Mucosal Proliferation

(Furukawa *et al.*, 2007) [14] It has been found that hyperplasia of the middle ear mucosa is a key factor in the development of otitis media and involves extensive cell proliferation and differentiation. Hyperplasia leads to tympanic effusion, mucus secretion production, and glue ear formation. Hyperplasia can also cause fibrosis of the tympanic structures to immobilize the auditory ossicular chain, and these injuries may occur in recurrent and/or chronic otitis media [15]. Mitogen-activated protein kinase (MAPK) cascades are known to cause altered gene expression and tissue proliferation in mammals, including extracellular signal-regulated kinase 1/2 (ERK1/ERK2), Jun N-terminal protein kinase (JNK), and p38 MAPK, with either ERK or p38 pathways reducing middle ear mucosal proliferation *in vitro* and p38 inhibition reducing bacterial-induced mucin gene expression in human middle ear epithelial cells. In animal models of OM, ERK activation did not correlate well temporally with ME mucosal hyperplasia because it occurred early and late in the OM. Mucosal growth was observed *in vitro* even at saturating levels of the MAPK kinase (MKK)/ERK inhibitor U0126 or the p38 inhibitor SB203580. This suggests that other pathways are involved in regulating proliferation. Upstream of the MKK kinase is another layer of JNK pathway protein kinases, such as the Rho GTPase family, in particular Rac and Cdc42. Further upstream, lipopolysaccharides, a component of the outer mem-

brane of Gram-negative bacteria (e.g., NTHI), stimulate the activation of JNK via Toll-like receptor 4 [14]. Inhibition of Rac/Cdc42, MLK, and JNK by themselves all reduced ME mucosal growth *in vitro*, suggesting that JNK activation via Rac/Cdc42 and MLK was activated in ME mucosal proliferation during OM.

3.2. HB-EGF (Heparin-Binding Epidermal Growth Factor)-Induced Mucosal Proliferation

HB-EGF was originally detected in the conditioned medium of the U937 macrophage-like cell line and was later found to be a member of the EGF growth factor family. HB-EGF is synthesized as a transmembrane protein (pro HB-EGF), which can be cleaved at the plasma membrane by a metalloproteinase to produce soluble HB-EGF (s HB-EGF). s HB-EGF exerts its mitogenic effect by binding and activating EGF receptor subtypes ErbB1 (a typical EGF receptor) and ErbB4 to exert its mitogenic effects. (Suzukawa *et al.*, 2014) [15] through the study of the mouse otitis media model, it was found that HB-EGF was expressed in a variety of tissues and a large number of cultured cells, including vascular endothelial and smooth muscle cells, inflammatory cells, skeletal muscle fibers, renal mesangial cells, keratinocytes, and tumor cells. Although normal tissues express relatively low levels of HB-EGF mRNA, increased expression is in response to various types of tissue damage, including hypoxia, injury, and partial excision. HB-EGF can also be upregulated in response to bacterial infection or stimulation of the LPS. HB-EGF is significantly involved in the proliferative response of the ME mucosa in the presence of bacterial OM. HB-EGF is one of the seven epithelial cell activity growth factors, and its expression kinetics are consistent with a role in mucosal proliferation. It is also the only factor that stimulates the growth of normal or previously infected mucosa *in vitro*. *In vivo* experiments confirmed the expression of the HB-EGF protein. The interaction of HB-EGF with its EGFR receptor plays a key role in ME mucosal proliferation in viral OM *in vivo* [16]. (Noel *et al.*, 2020) [1] It was demonstrated that heparin-bound EGF (HB-EGF) strongly stimulated the growth of cultured ME epithelial cells. In addition, the expression of HB-EGF in ME increased 26-fold during OM.

3.3. The NF-κB Signaling Pathway Accelerates ME Mucosal Growth

NF-κB is a dimeric transcription factor that is a pleiotropic regulator of many immune and inflammatory response processes, both in response to injury and infection and in promoting cell proliferation and survival, mediated through cell cycle regulation and growth factor stimulation. NF-κB plays a key role in inflammation, survival, stress response, and the cell cycle. There are five NF-κB family members in mammals: RelA/p65, RelB, c-Rel, p50 (NF-κB1), and p52 (NF-κB2). Different dimeric combinations of these subunits can be formed, and the heterodimer p50/p65 is commonly known to induce pro-inflammatory gene expression [17]. The NF-κB signaling pathway consists of a classical pathway and a non-classical or alternatively activated pathway. The classical pathway can be initiated by activated growth factor receptors (GFRs) via type I phosphatidy-

linositol 3-kinases (PI3Ks) and AKTs, TNF-α via its cognate receptor (TNFR1), IL-1 via the IL1 receptor (IL1R), or by innate immune receptors, including TLRs or NLRs, activating the expression of a number of genes involved in inflammation and/or tissue growth [3]. In the nonclassical pathway, 19 other members of the TNF receptor family phosphorylate NF-κB-inducible proteins (NIK) in response to stimulation by various ligands, inducing the expression of predominantly, but not exclusively, homologous immune-related genes. Classical activation preferentially regulates inflammation, proliferation, cell survival, and innate immunity genes. The nonclassical pathway primarily regulates lymphoid organ formation and B-cell maturation-related genes [18].

3.4. Involvement of the PI3K/AKT/PTEN Pathway in ME Mucosal Proliferation during OM

The PI3K/AKT pathway is an important regulator of cell growth in many systems and remains unexplored in ME. The PI3K (phosphatidylinositol 3-kinase) family consists of classes 1, 2, and 3 kinases that phosphorylate phosphatidylinositol bisphosphate (PIP2) to PIP3. Class 1 PI3K kinases are involved in the activation of AKT (protein kinase B), while classes 2 and 3 PI3Ks have other downstream targets. Inhibitors of PI3K/AKT/MTOR: Inhibitors of protein kinase B (AKT), phosphatidylinositol 3-kinase (PI3K), and mammalian target of rapamycin protein (MTOR) reduced exosome growth. Activation of AKT by PI3K occurs downstream of the action of many growth factor receptors and has long been associated with cell proliferation. Inhibition of AKT was found to reduce the growth of uninfected and previously infected ME mucosal explants [1]. AKT inhibition also reduced mucosal proliferation *in vivo* in OM. Pik3 and Akt genes were upregulated during OM, and AKT inhibition reduced ME mucosal proliferation. Cell growth promoted by activation of chemokine receptors CXCR1 and CXCR2 is mediated by AKT. (Lee *et al.*, 2020) [12] Two potential pathways mediating OM mucosal proliferation were identified: the PI3K/AKT pathway is a classical stimulant of tissue growth and cell survival. Typically, inhibitory PTEN appears to paradoxically mediate ME cell growth during OM, independently of its negative regulation of PI3K, through yet undefined processes. Inhibition of AKT and possibly PTEN may have the potential to reduce the pathophysiologic sequelae of this common childhood disease.

3.5. TLR Expression in Otitis Media

TLRs are a class of proteins that play a key role in the innate immune system. TLRs are found in immune cells such as dendritic cells, macrophages, neutrophils, T cells, and B cells, as well as in non-immune cells (e.g., fibroblasts, epithelial cells, and melanocytes). These TLRs are a major defense against infection. These TLRs are a major defense against infection, and Toll-like receptors enable the host to recognize a wide range of pathogen-associated molecular patterns, such as bacterial lipopolysaccharides, viral RNA, CpG-containing DNA, and flagellin. Several studies have shown that Toll-like receptors are a major compo-

nent of the microbial defense system [17] [19] [20]. TLR expression is absent or weak in normal middle ear mucosa but is increased in inflammatory fluid in the AOM, effusion in the OME, and granulation tissue and cholesteatoma in the COM. In addition, TLR showed increased or decreased expression depending on the presence of bacteria, disease recurrence, tissue type, and repeat surgery. In conclusion, TLR expression is associated with otitis media, and inappropriate TLR expression, or delayed or absent induction, is associated with the development, recurrence, chronicity, and complications of otitis media [8]. TLR-4 is a host cell expression pattern recognition receptor involved in a variety of functions, including the detection of bacterial endotoxin, with an opposite expression gradient. Thus, TLR-4 expression is greatest at the distal end of the Eustachian tube, where this anatomical site is usually considered sterile [21]. (Jung *et al.*, 2021) [20] has been shown that TLR4 is not expressed in normal middle ear specimens but is readily detected in COM and cholesteatoma. A comparison of TLR4 expression in normal ear canal skin, COM, and cholesteatoma showed elevated levels of TLR4 mRNA and protein in the mucosa and granulation tissue of patients with COM and cholesteatoma compared to normal external ear canal skin. In (Wang *et al.*, 2015) [22] study, TLR-2 and TLR-4 expression were found to be significantly increased in chronic rhinosinusitis without nasal polyps. (Hauber *et al.*, 2005) [23] the study confirmed that the number of TLR4 immunoreactive cells in the submucosa of bronchial biopsy tissue from cystic fibrosis (CF) lungs was increased and TLR4 expression in bronchial epithelial cells was decreased. Decreased TLR4 expression in epithelial cells may lead to the loss of a major defense mechanism of innate immunity, which contributes to chronic infection with Gram-negative bacteria. Reduced TLR2 expression in CF epithelial cells may lead to impaired innate defense mechanisms against Gram-positive bacteria. Increased numbers of TLR4-expressing cells in the submucosa of the CF airway provide inflammatory cells in response to Gram-negative bacteria, such as Pseudomonas aeruginosa, which is commonly found in the CF lung. Numerous studies have shown that HMGB1 is upregulated in the nasal mucosa in patients with chronic rhinitis with or without nasal polyposis. The middle ear is part of the nasopharyngeal tube unit, so it can be hypothesized that middle ear disease is also characterized by HMGB1 upregulation and inflammatory infiltration. The middle ear, nasal cavity, and bronchial tubes are part of the same respiratory epithelium and have similar cellular structures, so we can hypothesize that TLR-2 and TLR-4 play an important role in the pro-inflammatory response to otitis media. Inhibiting the inflammatory effects of TLR2 and TLR4 may be conducive to alleviating the pathophysiology of OM, and the inhibitors of TLR2 and TLR4 may be effective therapeutic drugs.

3.6. Expression of the High Mobility Group Protein 1 Gene (HMGB1) in Otitis Media

The high mobility group protein 1 gene (HMGB1) is an intranuclear non-histone protein that plays an important role in mediating the link between natural and

acquired immunity; when released extracellularly, it orchestrates the cellular stress response (in the necrotic state, stimulated by bacterial lipopolysaccharide) and serves as a marker of inflammation and as a cytokine. Extracellular HMGB1 acts as an immune adjuvant and triggers a strong response in T cells, dendritic cells, and endothelial cells. In addition, activated immune cells and endothelial cells also secrete HMGB1, creating a positive feedback loop that causes the release of additional cytokines and chemokines. All these properties make HMGB1 a key molecular target in a variety of human diseases, including sepsis, ischemia, immune disorders, neurodegenerative diseases, metabolic disorders, and cancer. (Lm *et al*., 2017) [24] study showed that HMGB1 was consistently nuclear-positive in epithelial cells from all of their samples (cholesteatoma, chronic otitis media, and otosclerosis); in inflammatory cells, HMGB1 was both nuclear and cytoplasmic-positive, but only the positive cells in the chronic otitis media samples were extracellularly-positive. HMGB1 is overexpressed in chronic middle ear lesions, and the entity of the expression is correlated with the degree of inflammatory response, suggesting that HMGB1 may play a critical role in the progression of inflammatory middle ear disease toward chronicity and more severe clinical manifestations. Identifying inhibitors of HMGB1 may have important therapeutic implications for all chronic inflammatory processes in the upper respiratory tract, including the middle ear. Among the HMGB1 inhibitors, glycyrrhetinic acid (GA) has been shown to be effective in adults and children with chronic inflammation of the upper respiratory tract with nasal congestion as the main symptom.

3.7. Effect of Lung Surface-Active Proteins (SPs) on Otitis Media

3.7.1. SP-A Modulates Middle Ear Mucosal Inflammation in Otitis Media

SPs are present on the luminal surface of lung epithelial cells, synthesized by alveoli from type II alveolar cells of the lung, and secreted into the alveolar lumen. Recent studies have reported the expression of the surface-active proteins SP-A and SP-D in porcine Eustachian tubes, SP-A in rabbit middle ear epithelial cells, and SP-D in rat middle ear epithelial cell lines [10] [25]. (Abdel-Razek *et al.*) [8] [25] [26] Several studies have shown that SP-A and SP-D are expressed in human middle ear and eustachian tube tissues and that SP-A and SP-D are also secreted by lung submucosal cells and Clara cells [8] [27]. SP-A Surface-active protein A (SP-A) is the most abundant surface-active protein in the lung and is an important component of the innate immune system. SP-A is a member of the collectin family of proteins that recognize carbohydrates on the surface of pathogens through a carbohydrate-recognition structural domain. As a pattern recognition receptor, SP-A functions as the first line of defense in the absence of specific antimicrobial antibodies [28]. As an important component of the natural immune system, it regulates cytokine production and increases the antimicrobial and antiviral functions of macrophages. SP-A regulates the inflammatory response induced by pathogen-derived products by binding to a variety of receptors, including Toll-like receptors 2 and 4. SP-A acts both as a conditioning

hormone and activates macrophage function, resulting in enhanced phagocytosis. In addition, SP-A has a variety of immunological effects, including stimulation of superoxide radical production and induction of chemotactic migration of macrophages. Due to its interaction with pathogens, it has also been suggested to play a role in hypersensitivity reactions and fungal infections. The presence of SP-A in the ME mucosa and its correlation with other pro-inflammatory cytokines suggest that SP-A plays a role in OM-phase neutrophil adhesion. Previous studies have demonstrated that SP-A is present in the eustachian tube of normal subjects, and (Li *et al.*) [27] [29] suggested that specialized cells in the Eustachian tube epithelium express SP-A and that pathogens may induce SP-A secretion into the Eustachian tube lumen during the inflammatory response to OM. In conclusion, SP-A promotes ME innate immunity by enhancing bacterial phagocytosis and killing and may regulate inflammation in the ME mucosa by modulating inflammation and NF-κB signaling activation [25].

3.7.2. SP-D Modulates Middle Ear Mucosal Inflammation in Otitis Media

SP-D is an antimicrobial protein that directly inhibits the proliferation of Gram-negative bacteria by increasing the permeability of microbial cell membranes in the form of macrophage phagocytosis and bacterial aggregation [8] [25] [26]. Expression of SP-D was increased in the human middle ear epithelial cell line (HMEEC-1) after attack with lipopolysaccharide in a LPS dose-dependent manner. Pattern recognition receptors (PRR) are important components of the innate immune system that help recognize pathogen-associated molecular patterns and eliminate virulence factors. The PRR induces the release and activation of cytokines to promote a range of immune responses to resolve infection. SP-A and SP-D belong to the group of C-type lectins, which function like the PRR and play an important role in host defense and the regulation of pulmonary inflammation [8] [25] [26]. Human SFTPD polymorphisms have been found to have an effect on SP-D protein assembly, function, and concentration and are associated with severe respiratory syncytial virus infections in humans, a known predisposing factor for OM. SP-D prevents TLR2 and TLR4 from interacting with both smooth and rough LPS, thereby inhibiting LPS-induced secretion of TNF-α by two serotypes of alveolar macrophages as well as the secretion of TNF-α by HEKMs expressing TLR4/MD-2 activation of NF-κB in HEK293 cells expressing MD-2 [26] [30].

4. Summary

Although surgical treatment is the most common method for the treatment of otitis media and has achieved good clinical efficacy, recurrent otitis media is not uncommon, especially in patients with cholesteatoma of the middle ear. Surgical treatment will also have a certain impact on the normal anatomical function of the middle ear, and the high complication rate is greater than the favorable impact on hearing loss. In recent years, more and more studies have shown that the natural immune function of the body is weakened, infectious factors are in-

creased, and the normal physiological function of the middle ear is affected, which may be closely related to the pathogenesis of otitis media. The normal middle ear is protected by the mucociliary system and innate immune secretion molecules, and the dysfunction of these defense mechanisms may be an important risk factor for the development of otitis media. During infection, these systems provide a critical protective barrier before adaptive immunity is activated. If defects in the disease mechanism can be identified, drug therapy would be an attractive alternative to existing surgical treatment; any immunosuppressant would be needed as an adjunct to antibiotic therapy; and a better understanding of the innate immunity of the middle ear may open up new avenues for the prevention and treatment of otitis media.

Conflicts of Interest

There are no interests and disputes in this article.

References

[1] Noel, J., Suzukawa, K., Chavez, E., *et al.* (2020) A Kinase Inhibitor Screen Identifies Signaling Pathways Regulating Mucosal Growth during Otitis Media. *PLOS ONE*, **15**, e0235634. https://doi.org/10.1371/journal.pone.0235634

[2] Liu, Z.D., Peng, F.S., Yan, M.S., *et al.* (2019) Incidence and Disease Burden of Otitis Media in China, 1990-2019. *Modern Medicine*, **51**, 1312-1319.

[3] Chen, Y. (1995) Smoking Environment (ETS) and Otitis Media. Foreign Medicine. *Journal of Otolaryngology*, No. 3, 172-173.

[4] Lee, H.M., Son, Y.S., Kim, H.S., *et al.* (2023) Effects of Particulate Matter Exposure on the Eustachian Tube and Middle Ear Mucosa of Rats. *Clinical and Experimental Otorhinolaryngology*, **16**, 225-235. https://doi.org/10.21053/ceo.2023.00227

[5] Machogu, E. and Gaston, B. (2021) Respiratory Distress in the Newborn with Primary Ciliary Dyskinesia. *Children* (*Basel*), **8**, Article No. 153. https://doi.org/10.3390/children8020153

[6] Murgasova, L., Jurovcik, M., Jesina, P., *et al.* (2020) Otorhinolaryngological Manifestations in 61 Patients with Mucopolysaccharidosis. *International Journal of Pediatric Otorhinolaryngology*, **135**, Article ID: 110137. https://doi.org/10.1016/j.ijporl.2020.110137

[7] Gu, Q.J. (2003) Research Progress on Pathological Changes of Eustachian Tube and Middle Ear Mucosa in Chronic Otitis Media. *Journal of Audiology and Speech Disorders*, No. 2, 144-146.

[8] Abdel-Razek, O., Audlin, J., Poe, D.S. and Wang, G. (2022) Surfactant Proteins and Innate Immunity of Otitis Media. *Innate Immun*, **28**, 213-223. https://doi.org/10.1177/17534259221123309

[9] Padurariu, S., Röösli, C., Røge, R., *et al.* (2019) On the Functional Compartmentalization of the Normal Middle Ear. Morpho-Histological Modelling Parameters of Its Mucosa. *Hearing Research*, **378**, 176-184. https://doi.org/10.1016/j.heares.2019.01.023

[10] Lim, D.J., Chun, Y.M., Lee, H.Y., *et al.* (2000) Cell Biology of Tubotympanum in Relation to Pathogenesis of Otitis Media—A Review. *Vaccine*, **19**, S17-S25. https://doi.org/10.1016/S0264-410X(00)00273-5

[11] Song, C.I., Hong, H.R. and Yoon, T.H. (2016) Influence of Middle Ear Mucosal Condition on Post-Tympanoplasty Audiologic Outcome. *European Archives of Oto-Rhino-Laryngology*, **273**, 581-585. https://doi.org/10.1007/s00405-015-3590-0

[12] Lee, H.H., Chin, A., Pak, K., Wasserman, S.I., Kurabi, A. and Ryan, A.F. (2020) Role of the PI3K/AKT Pathway and PTEN in Otitis Media. *Experimental Cell Research*, **387**, Article ID: 111758. https://doi.org/10.1016/j.yexcr.2019.111758

[13] Palacios, S.D., Pak, K., Rivkin, A.Z., *et al.* (2004) Role of p38 Mitogen-Activated Protein Kinase in Middle Ear Mucosa Hyperplasia during Bacterial Otitis Media. *Infection and Immunity*, **72**, 4662-4667. https://doi.org/10.1128/IAI.72.8.4662-4667.2004

[14] Furukawa, M., Ebmeyer, J., Pak, K., *et al.* (2007) Jun N-Terminal Protein Kinase Enhances Middle Ear Mucosal Proliferation during Bacterial Otitis Media. *Infection and Immunity*, **75**, 2562-2571. https://doi.org/10.1128/IAI.01656-06

[15] Suzukawa, K., Tomlin, J., Pak, K., *et al.* (2014) A Mouse Model of Otitis Media Identifies HB-EGF as a Mediator of Inflammation-Induced Mucosal Proliferation. *PLOS ONE*, **9**, e102739. https://doi.org/10.1371/journal.pone.0102739

[16] Sakamoto, T., Pak, K., Chavez, E., Ryan, A.F. and Kurabi, A. (2022) HB-EGF Plays a Pivotal Role in Mucosal Hyperplasia during Otitis Media Induced by a Viral Analog. *Frontiers in Cellular and Infection Microbiology*, **12**, Article ID: 823714. https://doi.org/10.3389/fcimb.2022.823714

[17] Ishizuka, E.K., Filgueiras, L.R., Rios, F.J., Serezani, C.H. and Jancar, S. (2016) PAFR Activation of NF-κB p65 or p105 Precursor Dictates Pro- and Anti-Inflammatory Responses during TLR Activation in Murine Macrophages. *Scientific Reports*, **6**, Article No. 32092. https://doi.org/10.1038/srep32092

[18] Cho, C.G., Pak, K., Webster, N., Kurabi, A. and Ryan, A.F. (2016) Both Canonical and Non-Canonical NF-κB Activation Contribute to the Proliferative Response of the Middle Ear Mucosa during Bacterial Infection. *Innate Immunity*, **22**, 626-634. https://doi.org/10.1177/1753425916668581

[19] Fransson, M., Adner, M., Erjefält, J., Jansson, L., Uddman, R. and Cardell, L.O. (2005) Up-Regulation of Toll-Like Receptors 2, 3 and 4 in Allergic Rhinitis. *Respiratory Research*, **6**, Article No. 100. https://doi.org/10.1186/1465-9921-6-100

[20] Jung, S.Y., Kim, D., Park, D.C., *et al.* (2021) Toll-Like Receptors: Expression and Roles in Otitis Media. *International Journal of Molecular Sciences*, **22**, Article No. 7868. https://doi.org/10.3390/ijms22157868

[21] Bakaletz, L.O. (2010) Immunopathogenesis of Polymicrobial Otitis Media. *Journal of Leukocyte Biology*, **87**, 213-222. https://doi.org/10.1189/jlb.0709518

[22] Wang, X., Zhao, C., Ji, W., Xu, Y. and Guo, H. (2015) Relationship of TLR2, TLR4 and Tissue Remodeling in Chronic Rhinosinusitis. *International Journal of Clinical and Experimental Pathology*, **8**, 1199-1212.

[23] Hauber, H.P., Tulic, M.K., Tsicopoulos, A., *et al.* (2005) Toll-Like Receptors 4 and 2 Expression in the Bronchial Mucosa of Patients with Cystic Fibrosis. *Canadian Respiratory Journal*, **12**, 13-18. https://doi.org/10.1155/2005/648984

[24] Bellussi, L.M., Vindigni, C., Cocca, S., *et al.* (2017) High-Mobility Group Box Protein 1 Expression in Inflammatory Diseases of the Middle Ear. *International Journal of Immunopathology and Pharmacology*, **30**, 168-173. https://doi.org/10.1177/0394632017698713

[25] Abdel-Razek, O., Ni, L., Yang, F. and Wang, G. (2019) Innate Immunity of Surfactant Protein A in Experimental Otitis Media. *Innate Immunity*, **25**, 391-400. https://doi.org/10.1177/1753425919866006

[26] Abdel-Razek, O., Liu, T., Chen, X., Wang, Q., Vanga, G. and Wang, G. (2021) Role of Surfactant Protein D in Experimental Otitis Media. *Journal of Innate Immunity*, **13**, 197-210. https://doi.org/10.1159/000513605

[27] Li, L., Guo, X., Olszewski, E., *et al.* (2015) Expression of Surfactant Protein-A during LPS-Induced Otitis Media with Effusion in Mice. *Otolaryngology—Head and Neck Surgery*, **153**, 433-439. https://doi.org/10.1177/0194599815587699

[28] Pettigrew, M.M., Gent, J.F., Zhu, Y., *et al.* (2006) Association of Surfactant Protein A Polymorphisms with Otitis Media in Infants at Risk for Asthma. *BMC Medical Genomics*, **7**, Article No. 68. https://doi.org/10.1186/1471-2350-7-68

[29] Li, G., Chen, T., Mao, Y., *et al.* (2023) Surfactant Protein A Can Affect the Surface Tension of the Eustachian Tube and Macrophage Migration. *Laryngoscope*, **133**, 1726-1733. https://doi.org/10.1002/lary.30396

[30] Yu, G.H., Kim, H.B., Ko, S.H., *et al.* (2018) Expression of Surfactant Protein-A in the *Haemophilus influenzae*-Induced Otitis Media in a Rat Model. *International Journal of Pediatric Otorhinolaryngology*, **112**, 61-66. https://doi.org/10.1016/j.ijporl.2018.06.030